

accompanied by the required reference to the sequence identifier.

Applicants have amended the specification to address these omissions. Applicants have submitted a new Figure 5 to indicate the sequence identifiers. Applicants defer submitting a new formal drawing for Figure 5 until Notice of Allowance is received. The anti-sense strand shown in Figure 5 was inadvertently omitted from the sequence listing as filed. Applicants have submitted a substitute Computer Readable Form and Paper Copy of the Sequence Listing to include this sequence.

35 U.S.C. § 112, second paragraph

The Examiner rejected claims 1-14 under 35 U.S.C. § 112, second paragraph, as being indefinite.

Claim 1 is rejected because the Examiner found that it was not clear how the sequence was modified. Applicants appreciate the suggestion, made by the Examiner, to adopt the formula presented on page 11 of the specification to clarify the claims. However, applicants have elected to amend claim 1 to address the issue raised by the Examiner. It is submitted that claim 1 as amended is clear.

The claims are rejected because the abbreviation "EPO" is used. As suggested by the Examiner, the applicants have amended the independent claims to the abbreviation has been spelled out to recite "Erythropoietin".

Claim 4, is rejected because there is no antecedent basis for "the linker sequence (GlyGlyGlyGlySer SEQ ID NO:123)" and also that SEQ ID NO:123 in the sequence

listing is "GlyGlyGlySer" not "GlyGlyGlyGlySer". Applicants have amended the claim to recite:

"polypeptide sequence (GlyGlyGlySer SEQ ID NO:123) in SEQ ID NO:1; SEQ ID NO:2; . . . and SEQ ID NO:122 is a [~~linker~~] polypeptide sequence selected from the group consisting of: . . .".

Applicants submit that as amend claim 4 is clear.

Claim 12, is rejected because it is not clear the meaning of "a factor" is. Applicants have amended claims 12 and 13, as suggested by the Examiner, to recite "a second protein".

Claim 13 is rejected because it contains an improper Markush group wherein the "and" is misplaced and the plural terms should be singular. Applicants have amended the claim 13 correct the noted errors.

Claim 14 is rejected because "patient" is misspelled. Applicants have amended claim 14 to correct the spelling error.

Applicants submit that the claims, as amended, are clear.

35 U.S.C. § 103

Claims 1, 5 and 10-14 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Pastan et al. (U.S. Patent 5,635,599) in view of Lin (U.S. Patent 4,703,008). The Examiner argues that it would have been obvious to make circular permuted EPO molecules having a breakpoint at positions 25, 27, 30, 32, 80, 82, 88, 116, or 121.

Applicants traverse this rejection. Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness for several reasons.

First, the entirety of the prior art fails to provide a reasonable expectation of success. To the contrary the prior art shows that there is a great deal of unpredictability associated with circular permutation.

At best, '599 is limited to the teaching of only two circular permutation breakpoints (37-38 and 104-105) of IL-4 in the context of a chimeric molecules with a cytotoxin or an antibody fragment (Fv). Only one circularly permuted form each of IL-2 (Example 5, column 25-26), G-CSF (Example 6, column 26) and GM-CSF (Example 6, column 26) are disclosed in '599. However, it is **not** shown that these IL-2, G-CSF, and GM-CSF molecules have any activity. Furthermore, it can also be taken from '599 that the circularly permuted ligands are useful when employed as a component in a chimeric molecule, not as a individual protein such as in the present invention. Accordingly, '599 specifically states the preferable use of two specific permutation molecules of IL-4 to partially restore bioactivity that was lost due to its context as a component of a chimeric protein with a cytotoxin or antibody. Therefore, the teaching of '599 can not be reasonable extended to other molecules besides IL-4. As admitted by the Examiner, '599 does **not** disclose a working example of circular permutation of Erythropoietin, nor do they disclose a sequence of Erythropoietin or

specifically include Erythropoietin in the generic list of molecules on column 4.

It is clear from prior art, as discussed in the present specification (pages 6-10), that the results of circular permutation have been highly variable. The applicants respectfully submit that the Examiner has failed to consider the **complete** teaching of the prior art. The totality of the prior art provides only a very limited number of examples of circular permuted proteins and the results have been variable. The primary motivation for many of these types of studies has been to study the role of short-range and long-range interactions in protein folding and stability. In many of the studies circular permutation disrupted the structure of the protein, and hence the bioactivity. The applicants point to numerous examples cited in the disclosure including; dihydrofolate reductase (Protasova et al., *Prot. Eng.* 7:1373-1377, 1995), Ribonuclease T1 Garrett et al., *Protein Science* 5:204-211, 1996, omp A (Koebnik & Krämer, *J. Mol. Biol.* 250:617-626, 1995), and yeast phosphoglycerate dehydrogenase (Ritco-Vonsovici et al., *Biochemistry* 34:16543-16551, 1995) pages 6-10, of circularly permuted molecules that have significantly lowered activity, solubility or thermodynamic stability. Clearly, due to the complex nature of the structure/function relationship, one skilled in the art would **not have a reasonably expectation of success** of predicting which if any of the presently claimed molecules would have the desired activity. This is also evident from '599 where it is pointed out that the modification of the ligand "represents a rearrangement of the molecule, neither the function, nor the desirability of such molecules was apparent prior to the work described

here" (transition sentence between column 1 and 2). Moreover, circular permutation is perhaps the most extreme form of protein engineering, involving the wholesale rearrangement of the linear amino acid sequence. One skilled in the art would recognize that circular permutation could have very extreme effects on the three dimensional structure of a protein and consequently could disrupt the complex structure/function relationship.

Second, the generic speculation in '599 about some general considerations for selecting breakpoints (columns 7 and 8) are not supported by the '599 specification. Clearly, Pastan is at best an invitation for experimentation not a basis for establishing a *prima facie* case of obviousness. It can not be concluded, from the limited disclosure and general speculation presented by Pastan about what may or may not be a good candidate for opening sites, that circular permutation is universally applicable to any given protein.

The Examiner specifically points to the speculation in '599 that a good choice for a opening site is where amino acid substitutions can be made. However, '599 does not provide a working example of a permutein that was made at a site where a substitution was made. The opening sites disclosed by '599 for IL-4 are limited to glycosylation sites. In '599 the nature of the reason for the selection of the opening site in IL-2, GM-CSF or G-CSF is not disclosed. The general guideline that sites at which substitutions can be made are good opening sites for circular permutation is only unsubstantiated speculation.

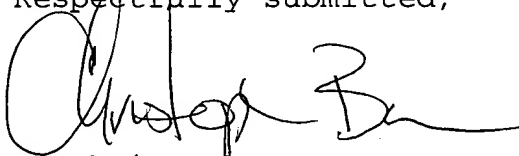
Third, the disclosure of '008 does not teach individual sites at which amino acid substitutions can be made. The Examiner cites the disclosure of '008 as providing sites where amino acid substitutions can be made in human Erythropoietin based on the alignment of human and cynomolgus monkey sequences (Figure 9). The sequence alignment shows that a total of fourteen residues are different between the two species. The applicants respectfully suggest that from this single alignment the Examiner has incorrectly concluded that, at any one of these single residue, amino acid substitutions can be made without effecting the bioactivity. However, it can only be properly concluded that all of the fourteen amino acid differences between the human EPO and cynomolgus monkey EPO are required. There is no teaching in '008 that any one of the single amino acid substitutions can be made without effecting activity. The Examiner rejected the claims because the cited breakpoints in the claims include positions 25, 27, 30, 32, 80, 82, 88, 116, or 121 which are among the positions at which human EPO and cynomolgus monkey EPO are divergent. It should also be noted from this sequence alignment using the same argument presented by the Examiner that positions 95, 99, 105, 139, and 163 would also good candidates for opening sites. To the contrary, the present application discloses that these are not good candidates for opening sites. The disclosure of '008 is limited to EPO molecules from two primate species and does not teach at which positions single amino acid substitutions can be made.

Regardless, even if sites were known at which substitutions could be made, the speculation in '599 that these would be good candidates is not supported by the '599

disclosure. As discussed above, the '599 application has failed to provide any evidence that opening sites can be made at positions at which substitutions can be made. It is further speculated in '599 that good candidates for opening sites are conserved sites between a family of related proteins. The disclosure of two species can hardly be considered a family of molecules from which it can be concluded which positions are conserved and which are not.

In conclusion, the applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness because; the prior art teaches that the results of circular permutation is unpredictable; the '599 application does support the speculation that breakpoints can be made at sites at which amino acid substitutions can be made; and that '008 does not disclose positions at which single amino acid substitutions can be made. Therefore, it has **not** be established that the prior art suggests the presently claimed molecules and the prior art does not provide a reasonably expectation of success.

Respectfully submitted,



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